MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2020

July–September 2020—Volume 135, pp. 471–489

https://doi.org/10.5248/135.471

Lepiota punaensis sp. nov. from Hawai'i Island, and a discussion of *L. elaiophylla*

Jeffery K. Stallman[1*], Don E. Hemmes[2], Nicole A. Hynson[3], Michael H. Shintaku[4]

[1*] *Department of Tropical Conservation Biology & Environmental Science Graduate Program, University of Hawai'i Hilo, Hilo, Hawai'i 96720 USA*

[2] *Department of Biology, University of Hawai'i Hilo, Hilo, Hawai'i 96720 USA*

[3] *Pacific Biosciences Research Center, University of Hawai'i Mānoa, Honolulu, HI 96822 USA*

[4] *College of Agriculture, Forestry, and Natural Resource Management and Department of Tropical Conservation Biology & Environmental Science Graduate Program, University of Hawai'i Hilo, Hilo, Hawai'i 96720 USA*

*Correspondence to: *jstall@hawaii.edu*

Abstract—A new species, *Lepiota punaensis*, in *L.* subsect. *Helveolinae* is described from the Puna District of Hawai'i Island. A species closely resembling *L. elaiophylla* in morphology but genetically distant from the species paratype is also discussed. Both taxa and additional Hawaiian collections are analyzed in an nrITS phylogeny, and a key to *Lepiota* on Hawai'i Island is given.

Key words—*Agaricaceae*, *Casuarina equisetifolia*, cryptic species

Introduction

Lepiota (Pers.) Gray is a large genus of more than 400 species (Kirk & al. 2008) with widespread distribution that continues to see new species described regularly from temperate (Caballero & al. 2015), subtropical (Qasim & al. 2015), and tropical (Sysouphanthong & al. 2016) environments. Exceptions occur, but in general the genus is characterized macroscopically by white to yellow-spored agarics with free gills, evidence of partial and universal veils, but lacking a volva. Microscopically the lamellar trama are regular, and

basidiospores are thin-walled, without a germ pore, generally dextrinoid, and not metachromatic in Cresyl Blue. *Lepiota* has historically been split into sections based on morphology (e.g., Bon 1993), and more recently, into clades based on molecular data (Vellinga 2003). *Lepiota* subsect. *Helveolinae* Bon & Boiffard [in *L.* sect. *Ovisporae* (J.E. Lange) Kühner], which contains the two species closely examined in this manuscript, is characterized morphologically by ellipsoid to oblong spores with a pileus covering composed of a trichoderm of predominantly long cylindrical elements and has been consistently recovered as monophyletic in molecular analyses of *Lepiota* and its infrageneric sections (Vellinga 2003; Qasim & al. 2015, 2016; Sysouphanthong & al. 2012, 2016; Liang & al. 2018; but see Liang & al. 2011). This clade contains species such as *Lepiota subincarnata* J.E. Lange and *L. brunneoincarnata* Chodat & C. Martín, which are known to contain amatoxins (Benjamin 1995).

Our project documenting the lepiotaceous fungi from Hawai'i Island reports a new taxon from *L.* subsect. *Helveolinae*, *Lepiota punaensis*, and a new record for Hawai'i, *Lepiota* cf. *elaiophylla*. In addition, *Lepiota aspera* (Pers.) Quél., *Lepiota besseyi* H.V. Sm. & N.S. Weber, *Lepiota pseudorubella* Gubitz, *Lepiota rubrobrunnea* Gubitz, and a poorly known taxon with an epithelial pileus covering (MK412575) are confirmed as occurring on Hawai'i Island. All species are found in wet, lowland environments associated with alien vegetation and are therefore presumed to be introduced.

Materials & methods

Collection and morphologic description

Basidiome collecting occurred from September 2016 to January 2019 on Hawai'i Island. Photographs were taken in the field with a Canon Rebel T4i camera and have not been altered (Plates 2a; 3a). Morphological details were recorded at the time of collection. Descriptions of Hawaiian habitats and vegetation zones are from Gagné & Cuddihy (1999). Macroscopic descriptions are based on terminology from Vellinga (2001) and color descriptions are from Kornerup & Wanscher (1978). After collection, specimens were desiccated before microscopic and molecular analysis, and are deposited at the Joseph F. Rock Herbarium, University of Hawaii at Mānoa, Honolulu, HI, USA (HAW).

Measurement of microscopic structures was performed on material rehydrated in 10% ammonia (pileus and stipe covering, spores), or 10% ammonia with 1% Congo Red (all other structures). Melzer's reagent, distilled water, and 1% Cresyl Blue in distilled water were also used to observe natural colors and color-changing reactions. Mature spores were measured in side view (excluding the hilar appendage) using the program Piximètre version 5.9 (http://ach.log.free.fr/Piximetre/). Measurements represent values falling within a 95% confidence

interval; the annotation [30, 4, 3] indicates that thirty spores from four specimens in three collections were measured. Micrographs for import to Piximètre were captured using an Olympus XM10 microscope camera attachment on an Olympus BX53 microscope with the software cell-sens Dimension version 1.5.

Molecular analysis

The primers ITS1F and ITS4 were used to amplify the nuclear ribosomal internal transcribed spacer region (nrITS; Schoch & al. 2012) via the polymerase chain reaction from dried fungal tissue of eleven specimens from Hawai'i Island. Amplicons were Sanger sequenced in both forward and reverse directions using the primers ITS1F and ITS4, and sequences were trimmed, edited, and forward and reverse reads aligned into consensus sequences in Geneious v. 9.1.8 (Kearse & al. 2012). Sequences generated were further investigated using the BLAST tool in GenBank (NCBI 2015), and the top sequences by similarity to Hawaiian taxa, as well

Table 1. Sequences newly generated and downloaded from GenBank for phylogenetic analysis.

taxon (collection #)	GenBank #	taxon (collection #)	GenBank #
C. hetieri	AY176459	*L. luteophylla*	AY176475
C. pulverulenta	AF391035	*L. magnispora*	AF391005
C. seminuda	JF907983	*L. pilodes*	AY176476
C. sp. (Hawai'i) (JKS 143)	**MK412600**	***L. punaensis* (JKS 64)**	**MK412572**
C. sp. (Hawai'i) (JKS 140)	**MK412604**	***L. punaensis* (JKS 86)**	**MK412577**
***Ch. molybdites* (JKS 96)**	**MK412591**	***L. punaensis* (JKS 87)**	**MK412578**
L. andegavensis	KP004931	***L. rubrobrunnea* (JKS 118)**	**MK412583**
L. apatelia	AY176462	*L. rhodophylla*	AY176480
L. aspera	KP843884	**L. sp. (O'ahu) (SS 1)**	**MK412617**
L. aspera	**MK412598**	*L.* sp. (Australia)	KP012693
L. brunneoincarnata	FJ998395	*L.* sp. (Australia)	KP012854
L. castanea	AY176463	**L. sp. (Hawai'i) (JKS 74)**	**MK412575**
L. castaneidisca	AF391061	**L. sp. (Kaua'i)**	**AY176402**
L. cristata	U85327	*L.* sp. (Thailand)	HQ647293
L.* cf. *elaiophylla	**MK412581**	*L.* sp. (Thailand)	JN224825
***L.* cf. *elaiophylla* (JKS 71)**	**MK412589**	*L.* sp. (Thailand)	JN224826
***L.* cf. *elaiophylla* (JKS 77)**	**MK412588**	*L.* sp. (Thailand)	JN224828
L. elaiophylla	MH979467	*L. subincarnata*	AY176491
L. elaiophylla	AF391024	*L. thiersii*	AY176492
L. erminea	AY176470	*L. tomentella*	EF080868
L. farinolens	AY176368	*L. vellingana*	HE974764
L. felina	U85330	*L. xanthophylla*	AY176405
L. helveola	MH979466	*M. haematospermum*	KF953545
L. himalayensis	HE614898	Uncultured fungus (Australia)	FJ528742

Sequences with collection numbers were newly generated; Hawaiian sequences are in **bold.**
C. = *Cystolepiota*, *Ch.* = *Chlorophyllum*, *L.* = *Lepiota*, and *M.* = *Melanophyllum*.

as representatives from each of the clades of *Lepiota* identified in Vellinga (2003) were included in our phylogenetic analyses (Table 1). Pairwise genetic distances (Table 2) were calculated in Geneious by comparing aligned, overlapping regions, excluding gaps and ambiguous positions. *Lepiota besseyi* was excluded from our phylogenetic analysis and key to *Lepiota* because it falls outside of *Lepiota* in the *Leucocoprinus* Pat. and *Leucoagaricus* Locq. ex Singer clade in *Agaricaceae* Chevall., although a formal transfer has not been made (Vellinga & al. 2011).

Table 2. Pairwise genetic distance comparison between *Lepiota* cf. *elaiophylla* and closest *Lepiota* relatives in GenBank.

	elaiophylla (Canada) MH979467	*elaiophylla* (Netherlands) AF391024	cf. *elaiophylla* (Hawai'i) MK412581	cf. *elaiophylla* (Hawai'i) MK412581	cf. *elaiophylla* (Hawai'i) MK412588
elaiophylla (Netherlands) AF391024	92.9%				
cf. *elaiophylla* (Hawai'i) MK412581	99.9%	92.9%			
cf. *elaiophylla* (Hawai'i) MK412589	99.9%	92.9%	99.9%		
cf. *elaiophylla* (Hawai'i) MK412581	99.9%	92.9%	99.9%	99.9%	
L. sp. (Australia) KP012854	98.3%	93.2%	98.3%	98.3%	98.3%

Sequences were aligned in Geneious with the default settings of the MAFFT (Katoh & Standley 2013) plugin, then imported to Molecular Evolutionary Genetics Analysis (MEGA) version X (Kumar & al. 2018). Based on the "Find Best DNA Model" tool in MEGA, Maximum Likelihood (ML) phylogenetic trees were created with all sites with gaps and/or missing data eliminated resulting in 465 total positions in the final dataset using the following settings: General Time Reversible (GTR) model (Nei & Kumar 2000) including gamma distributed (G) and invariant sites (I), moderate branch swap filter, and Subtree-Pruning-Regrafting (Extensive, level 5) for the ML heuristic method. The initial tree was built using the default neighbor-joining/bioneighbor-joining method, and the phylogeny was tested with 1000 bootstrap replicates.

The alignment was further analyzed using the Geneious plugin MrBayes (Huelsenbeck & Ronquist 2001). The tree was constructed with a GTR model including G and I sites with default settings (four chains for 1,100,000 generations, sampled every 200 generations with the first 100,000 generations discarded as burn-in). Tree images were edited in Inkscape version 3 (https://inkscape.org/).

Results & discussion

Our Bayesian and ML phylogenetic analyses generated identical tree topologies when considering our ML tree with the highest log likelihood (–4599.22) shown in Fig. 1. The topology of our *Lepiota* phylogeny is similar to prior analyses from the last 16 years and does not differ greatly from Vellinga's (2003) work using nrITS and nrLSU sequences, Qasim & al.'s (2015; 2016) works using nrITS sequences, Sysouphanthong & al.'s (2012; 2016) works using nrITS sequences, and Liang & al.'s (2018) work using nrITS, nrLSU, IGS, and mtSSU sequences (but see Liang & al. 2011 using nrITS, nrLSU, IGS, and mtSSU sequences where species with a trichoderm of long elements in clade 2 were dispersed).

Bootstrap support values for clades 2 and 4 (as defined by Vellinga 2003) are too low to draw general conclusions about the deeper relationships of our Hawaiian taxa within the genus, but our species clusters do reveal interesting information about close relatives of Hawaiian *Lepiota* s.l. species.

Lepiota punaensis is always on a long branch by itself with the closest nrITS match in GenBank an 87% similar Australian sequence (KP012854). *Lepiota punaensis* is sister to the cluster of species from Hawai'i, Canada, Australia, and Europe, all of which (except the Australian collection) are morphologically identified as *L. elaiophylla*. The Hawaiian *L.* cf. *elaiophylla* is genetically identical (99.9% similar, Table 2) to a collection identified as *L. elaiophylla* from a greenhouse in Canada, close to (98.3% similar, Table 2) a collection from a native *Eucalyptus* and *Acacia* swamp in Australia (M. Barrett, pers. comm.); these three geographically separated collections form a clade sister to the paratype of *L. elaiophylla* from the Netherlands, where the species was originally described. The collection from the Netherlands is 92.9% similar to the Hawaiian collections based on our alignment (Table 2).

Regarding other Hawaiian collections with a trichoderm of predominantly long elements, *L. rubrobrunnea* is on a long branch in a poorly supported group and its closest relatives are unclear. A *Lepiota* sp. collected on the UH Mānoa Campus on O'ahu clusters in a well-supported clade with *L. vellingana* Nawaz & Khalid, currently known from Pakistan (Nawaz & al. 2013), and *L. farinolens* Bon & G. Riousset, from France (Bon 1992). A species collected on the Island of Kaua'i clusters in a well-supported clade with three taxa from Thailand and *L. subincarnata*, a species known from Europe, North America, and South Asia (Razaq & al. 2014). It may be conspecific with sequence JN224826, a species collected in Chiang Mai Province in 2008 that, according to notes in GenBank, is "close to *L. subincarnata*."

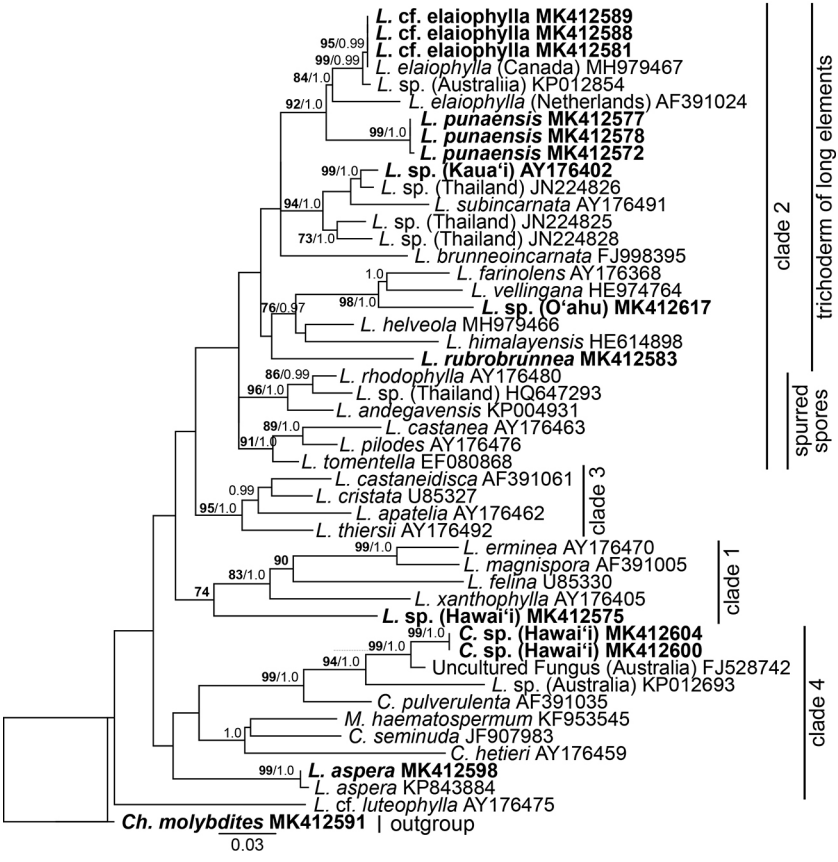

Fig. 1. Phylogenetic analysis of selected sequences and species of *Lepiota* based on nrITS sequences. The phylogeny was inferred using the Maximum Likelihood method and is drawn to scale, with branch lengths measured in the number of substitutions per site. Maximum Likelihood bootstrap support values ≥70% are shown in bold, and Bayesian posterior probabilities ≥0.95 are shown in normal type. Hawaiian taxa are in bold, and islands are indicated for taxa not identified to species. *C.* = *Cystolepiota*, *Ch.* = *Chlorophyllum*, *L.* = *Lepiota*, and *M.* = *Melanophyllum*.

The Hawaiian *Cystolepiota* species clusters in a well-supported clade with two unknown taxa from Australia, and these are sister to *Cystolepiota pulverulenta* (Huijsman) Vellinga [= *C. petasiformis* (Murrill) Vellinga], a purportedly widespread species found in Europe (Vellinga 2001), western North America (Vellinga 2006), and India (Kumar & Manimohan 2009). The closest Australian sequence (FJ528742) is 96% similar to the Hawaiian

Cystolepiota species, and its lack of any closer matches in GenBank and association with native vegetation supports the inference that it is a Hawaiian endemic (Hemmes & Desjardin 2002). A poorly known *Lepiota* (MK412575) that was only collected once in this study is on its own long branch in clade 1 (with only 74% support), and its closest relatives are unclear.

There are few data at this time to support broader conclusions about the origins and full ranges of Hawaiian *Lepiota* s.l. taxa. *Lepiota pseudorubella* lacks molecular data, *L. rubrobrunnea* and *L.* sp. (MK412575) have uncertain placement in our tree due to low support values, and *L.* sp. (MK412617) is on a relatively long branch in a clade with dispersed taxa. *Lepiota aspera* and *Melanophyllum haematospermum* (Bull.) Kreisel (for which no Hawaiian sequences are available) have widespread distributions, and *L. pseudorubella*, *L. rubrobrunnea*, and *L.* cf. *elaiophylla* have all been collected in artificially warm environments in temperate areas such as Canada and Germany (Gubitz 2008). The only putatively native Hawaiian taxon, *Cystolepiota* sp., is likely of Australian origin, while a collection from Kaua'i (AY176402) clusters with several species from Thailand. *Lepiota punaensis* and *L.* cf. *elaiophylla* have a close relative found in native Australian vegetation, but this is far from conclusive in establishing a location of origin or theory of dispersal.

Taxonomy

Lepiota punaensis Stallman, **sp. nov.** PLATE 2

MB 832104

Differs from *Lepiota elaiophylla* by its white lamellae and from *L. subincarnata* by its pileus covering containing short and narrowly clavate cells.

TYPE: USA, Hawai'i, Hawai'i Island, Puna District, MacKenzie State Recreation Area, 19°26′11″N 154°51′57″W, in duff under *Casuarina equisetifolia* L. and *Cocos nucifera* L., 9 Dec 2016, coll. JKS 86 (**Holotype**, HAW-F-00256, GenBank: MK412577).

ETYMOLOGY: from the Puna District of Hawai'i Island, the only known locality of this species.

PILEUS 8–24 mm broad, at first hemispherical, becoming obtusely conical to obtusely broadly conical, then convex to plano-convex in age; pileus covered in a dense mat of warm brown (6E6 to 7E4–5; occasionally 7F6) fibrils when young, quickly breaking into minute to small scales, composed of generally appressed aggregated fibrils, over a white to off-white (6B2) background as pileus expands, fibrils denser towards the cracked, or remaining intact disc surface, lessening towards margin, margin aspect straight, shape entire to eroded in age, often with remnants of the pileus covering adhering when young, dry, non-hygrophanous; CONTEXT thin (≤3 mm), white, unchanging;

LAMELLAE, L = 36, l = 1–3, free, close, subventricose to ventricose, ≤3 mm broad, white to off-white, edge even to eroded in age; STIPE 14–35 × 1–3 (–4 at base) mm, cylindrical, equal to tapering upwards, off-white to light brown (6B2, 8C2), covered with aggregated fibrils of similar appearance to pileus covering on lower 1/2 to 1/3 of stipe that are easily removed and become less conspicuous in age, appearing glabrous above this zone, but usually with thin covering of white hyphal strands oriented parallel to the stipe (lens), also easy to remove; stipe darkening over time to brown (6F5) where handled; CONTEXT thin, concolorous with exterior of stipe, stuffed with white hyphae when young, hollow in age, base equal to slightly enlarged; BASAL TOMENTUM absent to inconspicuous; RHIZOMORPHS absent or present, white; ANNULUS present only as annular zone; SPORE DEPOSIT white; EDIBILITY toxic, likely contains amatoxins; ODOR none to fungal; TASTE none to mildly fungal.

BASIDIOSPORES [94, 6, 4] (5.4–)6.3–6.6(–7.5) × (3.2–)3.7–3.8(–4.4) µm [x = 6.4 × 3.8 µm], Q = (1.4–)1.6–1.8(–2.0) [Qm = 1.7], ellipsoid to subcylindrical (generally oblong) in side view, usually with a straight adaxial (suprahilar depression absent) and convex abaxial side; oblong to rarely obovoid in frontal view, hyaline in H_2O and NH_4OH, dextrinoid, congophilous, weakly cyanophilous, non-metachromatic in Cresyl Blue, germ pore absent, mono to less commonly biguttulate; BASIDIA [63, 6, 4] (21.2–)25.6–33.3(–34.7) × (5.9–)6.5–8(–8.3) µm [x = 28.9 × 7.2 µm], hyaline, thin-walled, narrowly clavate to subclavate, sometimes with flexuous wall, with 4 sterigmata; LAMELLAR-EDGE sterile; CHEILOCYSTIDIA [44, 6, 4] (15.6–)22.3–24.6(–31.3) × (5.1–)8.9–10.2 (–14.0) µm [x = 23.5 × 9.5 µm], narrowly clavate to more commonly clavate or broadly clavate, hyaline, thin-walled; PILEUS COVERING an intricate trichoderm of long and shorter inflated cylindrical, sometimes flexuous, golden-colored hyphae with parietal pigment, thin to slightly thick-walled, shorter elements are present and common, but not in a distinctive layer, terminal elements narrowly clavate, cylindrical, inflated cylindrical, or less commonly irregular/flexuose narrowly clavate, or with a mucronate tip, ≤160 µm long and 12 µm diam, clamp connections common; STIPITIPELLIS a cutis of tightly packed hyaline, narrowly cylindrical hyphae ≤5 µm diam, scales below annular zone similar to those on pileus but generally composed of shorter elements, above annular zone irregularly present and mostly composed of long, narrowly cylindrical, hyaline, clamped hyphae; ANNULUS as zone only; CLAMP CONNECTIONS present in all tissues.

ADDITIONAL SPECIMENS EXAMINED—**USA**, **Hawai'i**, **Hawai'i Island**, **Puna District**, MacKenzie State Recreation Area, 19°26′11″N 154°51′57″W, 17 Sep 2016, coll. JKS

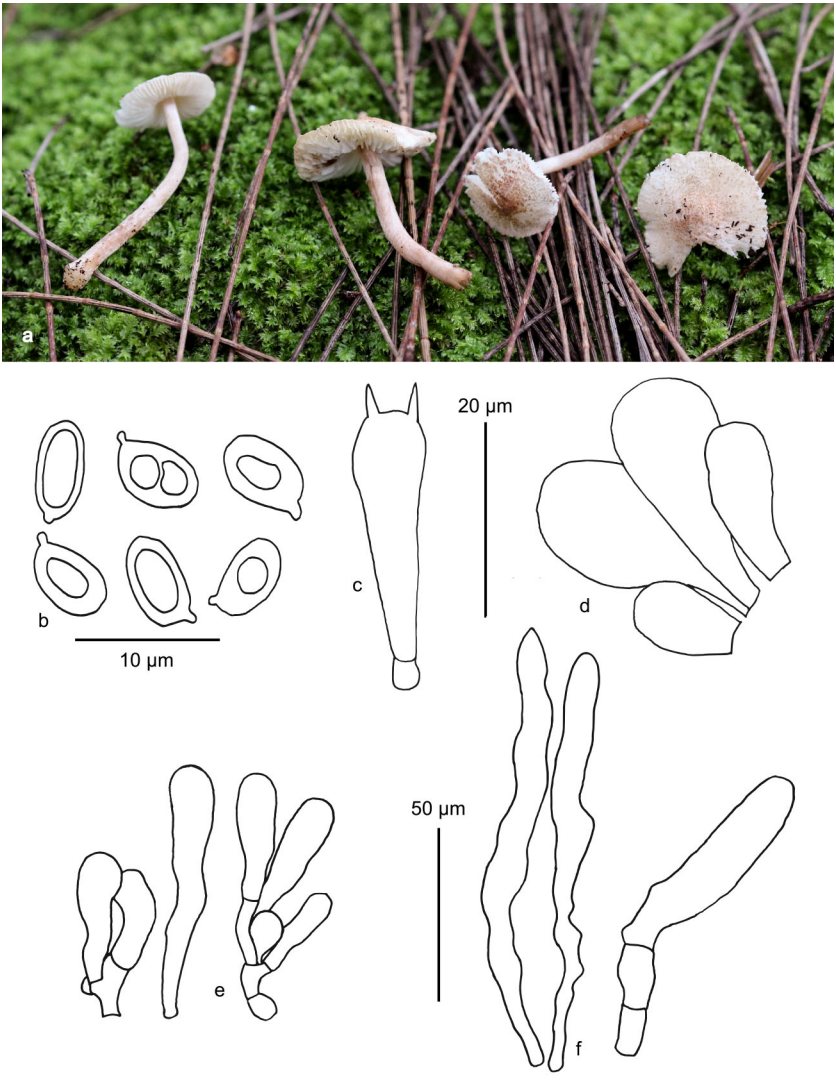

Plate. 2. *Lepiota punaensis* (holotype, HAW-F-00256). a. Basidiomata; b. Basidiospores; c. Basidia (with four sterigmata; only two shown in this basidial view); d. Cheilocystidia; e. Stipe covering; f. Pileus covering.

64 (HAW-F-00257; GenBank MK412572); 5 Jan 2017, coll. JKS 87 (HAW-F-00258; GenBank: MK412578); 1 February 2018, coll. JKS 186 (HAW-F-00255); 2 January 2019, coll. JKS 204 (HAW-F-00259).

Ecology & distribution — Solitary to scattered on needles and seed pods of *Casuarina equisetifolia* in wet, lowland, alien environments. January, February, September, and December. Currently known only from the Puna District of Hawai'i Island.

Comments—*Lepiota punaensis* is known only from *Casuarina equisetifolia* duff in the Puna District of Hawai'i Island. The species is recognized by its small size, lack of an annulus, and small, appressed brown fibrils over an off-white cap context. Its oblong spores and pileus covering of long elements without a layer of shorter clavate elements (although shorter elements are present) place this species in *L.* subsect. *Helveolinae* with other species that contain amatoxins.

Although *L. punaensis* is a small, relatively nondescript species, few described species provide similar morphological matches, and its nrITS sequence is unique. Recent studies from around the world such as Thailand (Sysouphanthong & al. 2012), China (e.g., Liang & al. 2018), Pakistan (Qasim & al. 2016), and the Dominican Republic (Justo & al. 2015) have described new *Lepiota* species from the (neo)tropics, and have included DNA sequence data that excludes any species from these studies, or other studies incorporating nrITS sequence data, from providing a match to *L. punaensis*. Older literature can be more difficult to interpret, but a review of species sharing similarities, and newly described species without molecular data also do not reveal a close match.

Lepiota zalkavritha T.K.A. Kumar & Manim. is a species of small size in *L.* sect. *Ovisporae* described from India with no nrITS sequence (Kumar & Manimohan 2009). *Lepiota zalkavritha* differs from *L. punaensis* in having occasional apical outgrowths on its cheilocystidia, being more robust, having darker brown coloration on pileus and stipe, and growth in soil (compared to *Casuarina equisetifolia* duff). *Lepiota brevipes* Murrill is a small species described from Florida in *L.* sect. *Ovisporae* with similar coloration to *L. punaensis*, but the stipe is often much wider, and the spores are smaller at 4.1–5 × 2.5–3.1 µm (Akers 1997). *Lepiota cinnamomea* Cleland is a small species from Australia in *L.* sect. *Ovisporae* (Aberdeen 1992), but the pinkish-brown and dark-brown color descriptions of the scales and disc, and shorter elements in the pileus covering (only ≤68 µm long) rule it out. Other small species described from Australia and stated to be in *L.* sect. *Ovisporae* (Grgurinovic 1998) have annuli (e.g., *L. rimosa* Murrill) or color-changing reactions not present in *L. punaensis*.

Pegler's work from Ceylon (1972) reveals species in *L.* sect. *Ovisporae* that are much larger, have unique red or yellow colors, or have annuli that do not match *L. punaensis*, and his works from Africa (1977) and the Caribbean (1983) do not reveal close matches. Rick's (1905, 1907, 1920, 1926, 1930, 1937, 1938, 1961) works from South America present few good matches for *L. punaensis* and Pereira's (1998, 2000, 2001) present none. Rick does describe a variety of *Lepiota* species with arachnoid veils (e.g., *L. flavipes* Rick and *L. hypholoma* Rick), but other features such as the coloration or spore sizes are different.

The closest morphological match appears to be *Lepiota subincarnata*, a well-known species described from Europe that is also found in North America and Asia (Razaq & al. 2014). *Lepiota punaensis* differs in having a typically smaller pileus and containing pileus covering elements that can have flexuose walls, be narrowly clavate, and contain shorter elements (although not in a distinctive layer), all of which *L. subincarnata* lacks.

Lepiota* cf. *elaiophylla Vellinga & Huijser PLATE 3

PILEUS (15–)25–40(–50) mm broad, at first paraboloid to narrowly hemispherical, then convex, expanding to plano-convex or applanate in age, completely covered in warm brown to dark brown (7F5, 8F4, 8F6, 8F8, 9F5) fibrils when young, quickly breaking up as the pileus expands into small to medium, generally appressed scales of aggregated fibrils over a dull yellow (3A4, 3B5, 4A3, 4A2) background; fibrils concentrated at disc (sometimes remaining unbroken) and gradually lessening towards margin to reveal more of surface; margin aspect straight, shape entire to eroded in age, sometimes with medium to large remnants of pileus covering adhering to margin, non-hygrophanous, dry; CONTEXT ≤5 mm thick, yellow (3A3, 3A4, 4B4, 4A3) fading to brown or reddish brown over time; LAMELLAE L = 32, l = 3 (usually), free, ventricose, up to 9 mm broad, dull yellow to sulphur yellow (3B6, 3B4), sometimes darkening in age (3A4, 4B4, 4C5); STIPE 20–40 × 3–7 mm, cylindrical (although sometimes flattening near the pileus) and equal (rarely tapering downwards), yellow (3A3, 3B4, 3A4, 4B3, 4A2, 3B4), with a clear annular zone beginning ⅔ to ¾ from the bottom of the stipe, above which the stipe appears glabrous, but remnants of the white, cortina-like partial veil generally remain, uncommonly aggregating into larger visible structures, below covered in medium to large, horizontally-oriented brown scales, generally appressed, but more uplifted than those on the pileus, concolorous with those on the pileus; CONTEXT fleshy-fibrous, color as in pileus or slightly lighter; when young solid to stuffed with translucent to

white hyphae, hollow in age; RHIZOMORPHS present, white; BASAL TOMENTUM generally present; SPORE DEPOSIT white; EDIBILITY toxic, likely contains amatoxins; ODOR none to mildly fungal; TASTE none to mild.

BASIDIOSPORES [150, 7, 5] (5.8–)7.0–7.2(–8.4) × (3.3–)3.7–3.8(–4.2) µm [x = 7.1–3.8 µm], Q = (1.5–)1.9(–2.3) [Qm = 1.9], narrowly ellipsoid to subcylindrical (usually oblong) in side view with a convex abaxial side and straighter adaxial side (mild suprahilar depression rarely present), oblong to subcylindrical in face view, hyaline in H_2O and NH_4OH, dextrinoid, congophilous, weakly cyanophilic to cyanophilic and non-metachromatic in Cresyl Blue, germ pore absent, mono- or (less commonly) bi-guttulate; BASIDIA [57,7,5] (20.6–)23.1–28.7(–34.6) × (5.7–)7.2–8.8(–9.2) µm [x = 26 × 8 µm], narrowly clavate to clavate, hyaline, thin-walled, 4-spored, rarely 2-spored; LAMELLAR-EDGE sterile; CHEILOCYSTIDIA [39, 5, 4] (15.5–)20.1–30.1(–39.3) × (5.2–)6.1–9.8(–10.3) µm [x = 24.5 × 7.9 µm], generally narrowly clavate to clavate, but also cylindrical and somewhat flexuous, sometimes with an apical extension, narrowly utriform, or less commonly subconical, often septate with one to two short, cylindrical basal cells, with or without clamp connections, hyaline, thin-walled; PLEUROCYSTIDIA absent; PILEUS COVERING an intricate trichoderm of mostly long erect and repent, generally cylindrical hyphae with brown intracellular and parietal pigments, lacking a layer of short clavate elements, terminal cells 25–240 × 4–15 µm, most commonly 60–100 µm in length, inflated cylindrical to narrowly clavate, often flexuous; STIPITIPELLIS a cutis of narrowly cylindrical hyaline hyphae 3–7 µm diam with stipe scales below annular zone similar to scales on pileus, above this zone a covering of thin, hyaline, cylindrical, often flexuous hyphae up to 4 µm diam and 100 µm long; ANNULUS as a cortinoid zone only; rarely with stipe covering elements aggregating to create a medial to superior raised region resembling an adhering "ring"; CLAMP CONNECTIONS present in all tissues; may be absent in cheilocystidia.

SPECIMENS EXAMINED—**USA**, **Hawai'i**, **Hawai'i Island**, **Hilo District**, University of Hawai'i Hilo Botanical Garden, 19°42′10″N 155°04′56″W, 28 September 2016, coll. JKS 69 (HAW-F-00244); **Hilo**, 'Imiloa Astronomy Center, 19°42′07″N 155°05′16″W, 5 October 2016, coll. JKS 71 (HAW-F-00245; GenBank MK412589); 6 November 2016, coll. JKS 77 (HAW-F-00246; GenBank MK412588); **Puna District**, MacKenzie State Recreation Area, 19°26′22″N 154°51′4″W, 15 November 2017, coll. JKS 174 (HAW-F-00247).

ECOLOGY & DISTRIBUTION—Scattered to gregarious, sometimes cespitose, in wood chips, on nutrient-rich soil in disturbed settings, or on *Casuarina equisetifolia* duff in wet, lowland, alien environments. January, May, and July

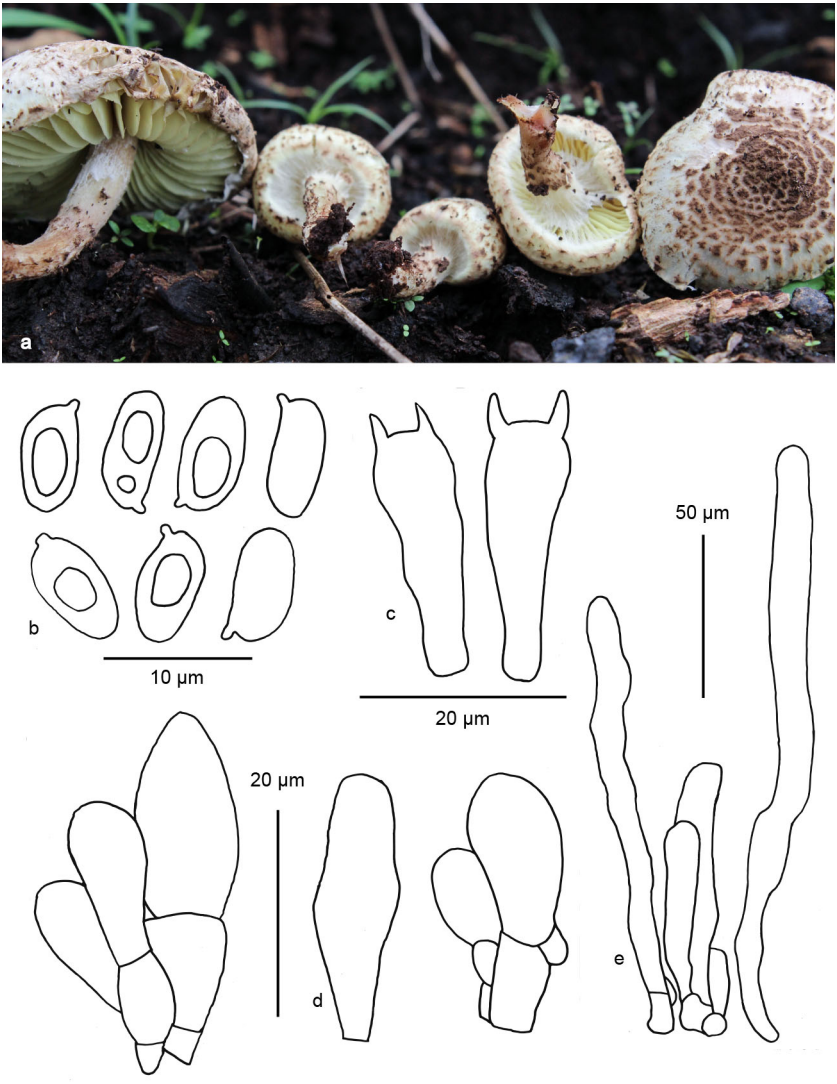

Plate. 3. *Lepiota* cf. *elaiophylla* (HAW-F-00246). a. Basidiomata; b. Basidiospores; c. Basidia (generally with four sterigmata; only two shown in these basidial views); d. Cheilocystidia; e. Pileus covering.

to December. Currently only known from the Puna and South Hilo Districts on Hawai'i Island; *L. elaiophylla* in the broad sense (see discussion) is reported

from Australia, Europe, North America, South America, India, and possibly Africa (as *L. xanthophylla* P.D. Orton).

Comments—*Lepiota* cf. *elaiophylla* occurs on wood chips, nutrient-rich soil in disturbed environments such as gardens, and under coastal *Casuarina equisetifolia* where it can fruit singly, or in large groups and cespitose clusters. Although many yellow lepiotaceous fungi occur in Hawai'i, *L.* cf. *elaiophylla* is easily distinguishable by its overall sulphur yellow coloration (including the gills) with brown scales on the pileus, robust stature (differentiating it from delicate yellow species in *Leucocoprinus*), and clear annular zone on the stipe with cortina-like partial veil remnants above a base covered in brown scales. Its oblong spores and pileus covering of primarily long elements place this species in *L.* subsect. *Helveolinae* with other species containing amatoxins.

A BLAST search reveals the top match by identity in GenBank to represent a collection identified as *Lepiota elaiophylla* by Else Vellinga, the co-describer of this species, from a greenhouse in Canada at 99.9% sequence similarity (KP012854). The next closest match (at 98.3% similarity) is MH979467 from a specimen collected near Darwin Australia growing in a native *Eucalyptus* and *Acacia* swamp habitat (M. Barrett, pers. comm.). Micrographs of the Australian collection (T. Lebel, pers. comm.) reveal spores that are more ellipsoid than in most collections of *L.* cf. *elaiophylla*, so it is unclear without additional Australian specimens whether these two taxa are morphologically identical.

Our phylogeny of *Lepiota* (Fig. 1) shows the Hawaiian *L.* cf. *elaiophylla* and the Canadian collection identified as *L. elaiophylla* as identical, with the Australian collection close on its own short branch. The Dutch paratype of *L. elaiophylla* is sister to the clade containing the Hawaiian, Canadian, and Australian collections, and a well-supported (84% bootstrap support, 1.0 Bayesian posterior probability) clade is formed when considering all taxa, including the paratype collection. See Table 2 for a comparison of pairwise genetic distances between the species discussed.

Lepiota elaiophylla was described from the Netherlands and is reported throughout Europe (e.g., Holec & Hálek 2008), Brazil (Wartchow & al. 2008, Ferreira & Cortez 2012), India (Kumar & Manimohan 2009), and possibly Africa (as *L. xanthophylla*; Pegler 1977). The Hawaiian *L.* cf. *elaiophylla* is clearly closely related to *L. elaiophylla,* from which it is macroscopically indistinguishable. The only differences are (morphologically) a more variable cheilocystidia shape in *L.* cf. *elaiophylla* and (molecularly) a different nrITS sequence, although some European collections outside the Netherlands and Brazilian collections show variation in cheilocystidia shape.

For a discussion of other *Lepiota* spp. with yellow lamellae (including the macroscopic lookalike *L. xanthophylla*, which is differentiated by containing a layer of short, clavate elements in the pileus covering and which has not been recorded from Hawai'i), see Vellinga & Huijser (1997). Collections of the European *L. xanthophylla* and *L. elaiophylla* are described with differently colored dried lamellae (yellow in *xanthophylla* and dirty olive-brown for *elaiophylla*). Collections of *L.* cf. *elaiophylla* generally have dark yellow lamellae when dried, although older, poorly dried, or damaged specimens may exhibit brown colors; so this does not appear to be a useful taxonomic feature at this time.

More information is needed to distinguish species concepts among *L.* cf. *elaiophylla*, the Australian collection, and *L. elaiophylla* from the Netherlands and throughout its reported range. One option is to argue that *L.* cf. *elaiophylla* falls within normal morphological variation (minor cystidia differences) of *L. elaiophylla*, a widespread species with high molecular variation and some morphological variation as evidenced by its presence in India (Kumar & Manimohan 2009), Brazil (Wartchow & al. 2008, Ferreira & Cortez 2012), and Australia. This option cannot be proven but falls on the conservative side of not describing a new taxon without first examining molecular data from other regions that might reveal whether there is a range of variation in the nrITS or two distinct taxa. The other option is to argue that *L.* cf. *elaiophylla* is clearly molecularly different from the Dutch *L. elaiophylla* (over 7% pairwise distance) and supported by a morphological difference (cheilocystidia shape). This argument is supported by pointing out that the related species *L. brunneoincarnata* and *L. subincarnata* initially showed little to no molecular variation across their respective geographic ranges (Europe and Central Asia; Europe, South Asia, and North America) when examined by Razaq & al. (2014).

At this time, more molecular data from Europe, South America, India (and possibly Africa) and morphological data from India and Africa are needed to clarify the species concept. The Indian collection was not given a full species description, but collections from South America reportedly have smaller spores as well as lageniform (Ferreira & Cortez 2012) or subfusoid (Wartchow & al. 2008) cheilocystidia, while collections from greenhouses in Europe primarily have narrowly clavate to clavate cheilocystidia (with the exception of the subglobose, and oval to pyriform cheilocystidia noted by Breitenbach & Kränzlin 1995). At this time, habitat does not seem to be a useful taxonomic feature as both Hawaiian and Dutch collections have been found in greenhouse

environments, although only the Hawaiian *L.* cf. *elaiophylla* is currently confirmed from non-artificial habitats in (sub)tropical environments.

Key to the *Lepiota* of Hawai'i Island

1. Pileus brownish with covering of acute pyramidal scales composed of globose or inflated elements 2
1. Pileus color variable (including brownish) with covering lacking acute pyramidal scales, at least some long (≥100 µm) cylindrical elements present 3
2. Basidiome with large membranous annulus, spores oblong to cylindrical, averaging 6.4 × 2.7 µm . . . . . *L. aspera*
2. Basidiome annulus and other features poorly known, spores ellipsoid to oblong, averaging 4.4 × 2.7 µm . . . . . *L.* sp. (MK412575; poorly known; see text)
3. Basidiome with ellipsoid spores averaging 5 × 3 µm 4
3. Basidiome with ellipsoid to oblong spores averaging 7 × 3–4 µm 5
4. Basidiome with prominent basal stipe ornamentation concolorous with pileus scales; farinaceous odor when crushed; pileus and stipe covering of long flexuose hyphae lacking abundant shorter clavate elements . . . . . *L. rubrobrunnea*
4. Basidiome generally lacking prominent basal stipe ornamentation, stipe scales present usually lighter than pileus scales; lacking a farinaceous odor when crushed; pileus and particularly stipe covering with abundant short clavate elements . . . . . *L. pseudorubella*
5. Basidiome with brown scales over a dull yellow pileus background, lamellae and stipe also yellow; arachnoid partial veil usually leaving only zone on stipe; pileus covering a trichoderm of long (≤240 µm) elements lacking shorter cells . . . . . *Lepiota* cf. *elaiophylla*
5. Basidiome with warm brown scales over an off-white pileus background lacking yellow coloration in all tissues; annulus a zone only; pileus covering a trichoderm of long (≤160 µm) elements with shorter, sometimes clavate hyphae interspersed but not in a distinct layer; shorter hyphae more common in stipe covering, currently known only from *Casuarina equisetifolia* duff in the Puna District of Hawai'i Island . . . . . *L. punaensis*

Acknowledgments

We would like to acknowledge the National Geographic Society, Puget Sound Mycological Society, and Sonoma County Mycological Society for funding. Thank you to Dr. Teresa Lebel and Dr. Matthew Barrett for sharing information on the Australian collection (GenBank KP012854), to Sean Swift for a collection made on O'ahu (GenBank MK412617), and to Dr. Else Vellinga on a variety of topics related

to *Lepiota* taxonomy in general and *L. elaiophylla* in particular. We also thank Dr. Brian A. Perry (Department of Biological Sciences, California State University East Bay, USA) and Dr. Alfredo Vizzini (Department of Life Sciences & Systems Biology, University of Torino, Italy) for presubmission review.

Literature cited

Aberdeen JEC. 1992. Lepiotoid genera (*Agaricales*) in south-eastern Queensland. Gailes, Queensland: Aberdeen Publications.

Akers B. 1997. The family *Lepiotaceae* (*Agaricales*, *Basidiomycetes*) in Florida. PhD dissertation. Southern Illinois University, Carbondale, IL.

Benjamin DR. 1995. Amatoxin syndrome poisoning by the amanitins. 198–241, in: Mushrooms poisons and panaceas. New York: W.H. Freeman and Company.

Bon M. 1992. Clé analytique des cortinaires alpins (avec quelques incursions dans la rhodoraie et l'aulnaie verte). Documents Mycologiques 22: 43–67.

Bon M. 1993 Flore mycologique d'Europe, 3. Les Lépiotes. *Lepiotaceae* Roze. Documents Mycologiques Mémoire hors série no. 3: 1–153. Lille, France: L'Association d'Ecologie et Mycologie.

Breitenbach J, Kränzlin F. 1995. Fungi of Switzerland. volume 4. Agarics 2nd part. Switzerland: Edition Mycologia Lucerne.

Caballero A, Vizzini A, Muñoz G, Marco C, Ercole E. 2015. *Lepiota elseae* (*Agaricales*, *Agaricaceae*), a new species of section *Lepiota* from Spain. Phytotaxa 201: 188–196. https://doi.org/10.11646/phytotaxa.201.3.2

Ferreira AJ, Cortez VG. 2012. Lepiotoid *Agaricaceae* (*Basidiomycota*) from São Camilo State Park, Paraná State, Brazil. Mycosphere 3: 962–976. https://doi.org/10.5943/mycosphere/3/6/11

Gagné WC, Cuddihy LW. 1999. Vegetation. 45–114, in: WL Wagner & al. (eds). Manual of the flowering plants of Hawai'i. Honolulu, HI: Bishop Museum Press.

Grgurinovic CA. 1998. Larger fungi of South Australia. Botanic Gardens of Adelaide and State Herbarium, Adelaide, Australia.

Gubitz C. 2008. Zwei neue *Lepiota*-Arten aus den Tropenhäusern des Ökologisch-Botanischen Gartens der Universität Bayreuth. Zeitschrift für Mykologie 74: 135–146.

Hemmes DE, Desjardin DE. 2002. Mushrooms of Hawai'i. Berkeley, California: Ten Speed Press.

Holec J, Hálek V. 2008. Record of the rare greenhouse fungus *Lepiota elaiophylla* (*Agaricales*, *Agaricaceae*) in Prague, Czech Republic, with notes on its taxonomy and distribution. Mycotaxon 105: 433–439.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Justo A, Angelini C, Bizzi A. 2015. Two new species and a new record of *Lepiota* (*Basidiomycota*, *Agaricales*) from the Dominican Republic. Mycological Progress 14(56): [9 p.]. https://doi.org/10.1007/s11557-015-1080-9

Katoh K, Standley DM. 2013. MAFFT Multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth & Bisby's dictionary of the fungi. 10th edition. CAB International, Wallingford, UK. https://doi.org/10.1079/9780851998268.0000

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S & al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. Copenhagen, Denmark: Politikens Forlag. https://doi.org/10.1093/bioinformatics/bts199

Kumar TK, Manimohan P. 2009. The genus *Lepiota* (*Agaricales*, *Basidiomycota*) in Kerala State, India. Mycotaxon 107: 105–138. https://doi.org/10.5248/107.105

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Liang JF, Yang ZL, Xu DP. 2011. A new species of *Lepiota* from China. Mycologia 103: 820–830. 10.3852/10-216. https://doi.org/10.3852/10-216

Liang JF, Yu F, Lu JK, Wang SK, Song J. 2018. Morphological and molecular evidence for two new species in *Lepiota* from China. Mycologia 110: 494–501. https://doi.org/10.1080/00275514.2018.1464333

Nawaz R, Khalid AN, Hanif M, Razaq A. 2013. *Lepiota vellingana* sp. nov. (*Basidiomycota*, *Agaricales*) a new species from Lahore, Pakistan. Mycological Progress 12: 727–732. https://doi.org/10.1007/s11557-012-0884-0

NCBI RC. 2015. Database resources of the National Center for Biotechnology Information. Nucleic Acids Research 44: D7–D19. https://doi.org/10.1093/nar/gkv1290

Nei M. Kumar S. 2000. Molecular evolution and phylogenetics. Oxford University Press, New York.

Pegler DN. 1972. A revision of the genus *Lepiota* from Ceylon. Kew Bulletin 27: 155–202. https://doi.org/10.2307/4117880

Pegler DN. 1977. A preliminary agaric flora of East Africa. Kew Bulletin Additional Series 6: 15–571.

Pegler DN. 1983. Agaric flora of the Lesser Antilles. Kew Bulletin Additional Series 9: 1–668.

Pereira AB. 1998. Espécies novas do gênero *Lepiota* (*Agaricaceae*) do sul do Brasil. Iheringia, Série Botânica. 51: 227–247.

Pereira AB. 2000. Contribuição ao conhecimento do gênero *Lepiota* no Brasil. I. Pesquisas, Série Botânica 50: 27–77.

Pereira AB. 2001. Contribuição ao conhecimento do gênero *Lepiota* no Brasil. II. Pesquisas, Série Botânica 51: 7–30.

Qasim T, Khalid AN, Vellinga EC, Razaq A. 2015. *Lepiota albogranulosa* sp.nov. (*Agaricales*, *Agaricaceae*) from Lahore, Pakistan. Mycological Progress 14(24): [6 p.]. https://doi.org/10.1007/s11557-015-1037-z

Qasim T, Khalid AN, Vellinga EC. 2016. A new species of *Lepiota*, *Lepiota lahorensis*, from Lahore, Pakistan. Turkish Journal of Botany 40: 419–426. https://doi.org/10.3906/bot-1507-31

Razaq A, Vellinga EC, Ilyas S, Khalid AN. 2014. *Lepiota brunneoincarnata* and *L. subincarnata*: distribution and phylogeny. Mycotaxon 126: 133–141. https://doi.org/10.5248/126.133

Rick J. 1905. Pilze aus Rio Grande do Sul. Annales Mycologici 3: 235–240.

Rick J. 1907. Contributio ad monographiam Agaricacearum et Polyporacearum Brasiliensium. Brotéria, Série Botânica 6: 65–92.

Rick J. 1920. Contributio ad monographiam Agaricacearum Brasiliensium. Brotéria, Série Botânica 18: 48–63.

Rick J. 1926. Descripção de algumas especies novas da mycoflora Riograndense. Egatea 11: 16–17.

Rick J. 1930. Contributio IV ad monographiam Agaricacearum Brasiliensium. Brotéria, Série Botânica 24: 97–118.

Rick J. 1937. Agarici riograndenses. Lilloa 1: 307–346.

Rick J. 1938. Agarici riograndensis. II. Lilloa 2: 251–316.

Rick J. 1961. *Basidiomycetes* eubasidii in Rio Grande do Sul, Brasilia, 5. Iheringia, Série Botânica 8: 296–449.

Schoch, CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA & al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences USA 109: 6241–6246. https://doi.org/10.1073/pnas.1117018109

Sysouphanthong P, Hyde KD, Chukeatirote E, Bahkali AH, Vellinga EC. 2012. *Lepiota* (*Agaricales*) in Northern Thailand – 2. *Lepiota* section *Lepiota*. Cryptogamie, Mycologie 33: 25–42. https://doi.org/10.7872/crym.v33.iss1.2012.025

Sysouphanthong P, Guo J, Hyde K, Xu J, Mortimer P. 2016. *Lepiota thailandica* (*Agaricaceae*), a new species from Thailand. *Phytotaxa* 245: 262–270.
https://doi.org/10.11646/phytotaxa.245.4.3

Vellinga EC. 2001. 4. *Macrolepiota*. 5. *Leucocoprinus*. 6. *Leucoagaricus*. 7. *Lepiota*. 8. *Chamaemyces*. 9. *Cystolepiota*. 64-162, in: ME Noordeloos & al. (eds). Flora Agaricina Neerlandica 5. Rotterdam, Netherlands: A. A. Balkema Publishers.

Vellinga, EC. 2003. Phylogeny of *Lepiota* (Agaricaceae)--Evidence from nrITS and nrLSU sequences. Mycological Progress 2: 305–322. https://doi.org/10.1007/s11557-006-0068-x

Vellinga EC. 2006. Lepiotaceous fungi in California, U.S.A. – 4. Type studies of *Lepiota fumosifolia* and *L. petasiformis*. Mycotaxon 98: 225–232.

Vellinga EC, Huijser HA. 1997. *Lepiota xanthophylla* and its greenhouse counterpart. Bollettino Gruppo Micologico G. Bresadola, n.s. 40: 457–464.

Vellinga EC, Sysouphanthong P, Hyde KD. 2011. The family *Agaricaceae*: phylogenies and two new white-spored genera. Mycologia 103(3): 494–509. https://doi.org/10.3852/10-204

Wartchow F, Putzke J, Cavalcanti MAdQ. 2008. *Agaricaceae* Fr. (*Agaricales*, *Basidiomycota*) from areas of Atlantic Forest in Pernambuco, Brazil. Acta Botanica Brasilica 22: 287–299. https://doi.org/10.1590/S0102-33062008000100026